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Clinical and Epidemiologic Characteristics of Respiratory Syncytial Virus Subgroups A and B Infections in Santa Fe, Argentina

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Respiratory Syncytial Virus (RSV) has two major antigenic groups, A and B. The implications of these variants in the epidemiology and pathogenesis of RSV infection are not well defined. This study was undertaken to compare the two RSV subgroups in patients admitted to hospital. Clinical and epidemiologic features of RSV subgroups in children under 30 months of age with proven RSV acute lower respiratory infections were examined during 4 winters from 1993 to 1996 in Santa Fe, Argentina. RSV typing was carried out with monoclonal antibodies in nasopharyngeal cells by indirect immunofluorescence. Of the 177 RSV positive nasopharyngeal aspirates obtained from 1993 to 1996, 85 (48%) were available for typing. Seventy-three (85.9%) specimens were identified as Subgroup A and 12 (14.1%) as Subgroup B. Except in 1993, in which only Subgroup A was detected, both variants circulated throughout the epidemic season. Subgroup A infections produced more severe disease than Subgroup B infections, as assessed by the length of the hospital stay and the use of respiratory support. This difference was age related, being evident in infants 0–6 months old. Patients with Subgroup B infections were also significantly less frequently breast-fed (95% vs. 75% for A and B subgroups, respectively; $P = 0.04$). It is concluded that the severity of disease in Argentinian patients admitted with acute RSV infections may be associated with Subgroup A strains as determined by a serogrouping method. ***J. Med. Virol. 61:76–80, 2000.***

KEY WORDS: RSV subgroups; respiratory disease; Argentinian children

INTRODUCTION

Respiratory Syncytial Virus (RSV) is the major viral pathogen of Acute Lower Respiratory Infections (ALRI) in children under five years of age who require hospitalisation. It is the main cause of bronchiolitis and pneumonia in children under six months of age [Avila et al., 1989; Weissenbacher et al., 1990; Celadilla et al., 1992; Sequeira et al., 1997; Videla et al., 1998].

With the development of monoclonal antibodies (Mabs) RSV isolates have been subdivided into two major antigenic groups (A and B) [Anderson et al., 1985; Mufson et al., 1985; Gimenez et al., 1986; Örvell et al., 1987]. Antigenic and genetic differences between both subgroups have been detected in several structural proteins. Some of these proteins play an important role in the infectivity and replication of RSV and the immune response to RSV and, therefore, their antigenic variation may have implications in the epidemiology and pathogenesis of RSV infections.

Published reports disagree as to whether or not there is a difference in the pathogenicity of the two RSV subgroups [Taylor et al., 1989; Monto and Ohmit 1990; Hall et al., 1990; McIntosh et al., 1993; Hornsleth et al., 1998]. If an association between severity of infection and RSV subgroups could be established, the early information about the RSV variant causing the disease might help the clinician to decide on therapy (like Ribavirin treatment), especially in younger infants.

Between 1993 and 1996, the Instituto Nacional de Enfermedades Respiratorias (INER) "E. Coni" and the Centro de Educación Médica e Investigaciones Clínicas (CEMIC) "N. Quirno" carried out a study to determine

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the epidemiology and etiology of ALRI in children under five years of age. RSV was the etiologic agent recognised most frequently in ALRI in Santa Fe City, accounting for 39.3% of bronchiolitis and 16.4% of pneumonia cases [Sequeira et al., 1997].

This study was undertaken to compare the virulence of the two RSV subgroups, A and B. We analysed clinical and epidemiologic features of RSV subgroups in children under 30 months of age with proven RSV infection and ALRI.

MATERIALS AND METHODS

Patients

Children under 30 months of age recently admitted to the "R. Gutierrez" paediatric Hospital, Santa Fe City, with presumptive diagnosis of bronchiolitis or pneumonia were studied. The study population was selected weekly (from Monday to Thursday) during the winters from 1993 to 1996. At admission, a complete clinical history, an informed consent signed by the parent and a nasopharyngeal aspirate (NPA) for viral diagnosis were obtained. The samples were collected into MEM (minimum essential medium) with bovine albumin, streptomycin and gentamycin. The NPAs were transported on ice to the INER Laboratory and processed immediately by indirect immunofluorescence for rapid viral diagnosis [Sequeira et al., 1997]. One hundred and seventy seven children with laboratory confirmed RSV infection were included in the analysis.

RSV Typing

Replicate fixed NPA smears were stored at −70°C and sent to CEMIC laboratory for RSV typing. A and B subgroups were characterised with Mabs by indirect immunofluorescence. Two Mabs against glycoprotein F were used. Mab 47F was specific for glycoprotein F from both subgroups whereas Mab 2F detects only Subgroup A. The reaction was revealed with a fluorescein labelled anti-mouse immunoglobulin (Sigma Chemical Co., St. Louis, MO). Readings were carried out with a C. Zeiss microscope fitted with epifluorescent equipment. The Mabs were produced by Dr. Melero in the Instituto Carlos Tercero, Madrid, Spain. Of the 177 RSV positive NPAs obtained from 1993 to 1996, only 85 (48%) were available for typing. In the remaining samples, no characterisation could be made due to scarce sample, lack of replicates or poor conditions of slide preservation.

Clinical Data

Clinical information abstracted from the clinical history was recorded before disclosure of the RSV subgroup. Data about age, grade of nutrition, perinatal respiratory pathology, prematurity (<38 weeks gestation), underlying pulmonary, cardiac or neurologic diseases, feeding history and days of evolution at admission were collected. As an indicator of clinical severity of disease, information about the requirement of supplementary oxygen, humid and lukewarm oxygen or artificial ventilation among hospitalised children was recorded. In addition, the length of the stay and the diagnosis on discharge were collected. Because some of the discharge diagnoses were not in agreement with the admission diagnoses, 11 children with RSV positive bronchitis and asthma were included in the study.

Statistical Analysis

For analysis in which dependent and independent variables were both categorical, χ^2 or Fisher's exact tests were used. For analysis in which the dependent variable was continuous, non-paired Student's *t*-test or Mann–Whitney tests were used. Because breastfeeding–RSV subgroups association could be related to the different mean ages of the groups, both factors were included in a multivariate logistic-regression model, with Subgroup A and B infection as dependent outcomes.

RESULTS

The characteristics of patients whose samples were not sub-grouped did not differ from patients with subgrouped NPAs (Table I). Of the 85 sub-grouped samples, 73 (85.9%) corresponded to Subgroup A and 12 (14.1%) were Subgroup B. Both variants were identified during all years, except in 1993, in which only Subgroup A was found (Table II). Clinical and demographic characteristics of infants with Subgroup A and Subgroup B infections are shown in Table III. Forty-eight of 73 children (63%) with Subgroup A infections were males, as compared with five of the 12 (42%) with Subgroup B infections; however, this difference was not statistically significant (Fisher's exact test, $P = 0.12$). The mean age for Subgroup B infections was greater than for Subgroup A, although it did not reach statistical significance (Student's *t*-test, $P = 0.63$). The relative frequencies of clinical categories of the illnesses caused by each subgroup were determined. Fewer children with Subgroup B infections developed bronchiolitis compared with children with Subgroup A infections (33% vs. 56% for B and A infections, respectively; Pearson Chi-squared, $P = 0.14$).

The interval between the onset of illness and admission to hospital were similar in both groups of patients (Mann–Whitney test, $P = 0.25$). The clinical severity of the illness was assessed by a clinical score using the following indications, each of which was assigned a score of one point if present and 0 if absent: 1) duration of the hospital stay >5 days; and 2) respiratory support given as oxygen therapy or assisted ventilation. A comparison of the subgroups with respect of severity of the disease, showed that A infections were significantly more severe than B infections (Mann–Whitney test, $P = 0.011$). This difference was also evident in the age group 0 to 6 months (Mann–Whitney test, $P = 0.033$). When two Subgroup A infected infants with congenital heart disease (a condition known to predispose to severe RSV infection) were removed from the analysis, the difference in severity between both subgroups was also confirmed (Mann–Whitney test, $P = 0.015$).

TABLE I. Comparison of RSV Infected Infants With and Without Subgroup Characterisation (Sante Fe, 1993–1996)

	Patients with RSV infection		
	Without subgroup characterisation (n = 92)	With subgroup characterisation (n = 85)	*P* value[a]
Gender			
Males:Females	50:42	53:32	0.35 (P)
Mean age (months) (SD)	5.28 (2.70)	4.62 (1.24)	0.30 (T)
Illness			
Bronchiolitis	47	45	0.45 (P)
Pneumonia	30	29	
Others (bronchitis, asthma)	15	11	
Days of evolution before admission (median)	4	3	0.9 (MW)
Days in hospital			
>5 days	25	18	0.45 (P)
Underlying conditions			
Yes	39	30	0.42 (P)
Breast-feeding history[b]			
Positive	62	74	0.32 (P)

[a]P = Pearson chi-squared, T = Student's *t*- test; MW = Mann–Whitney test.
[b]There were 85 and 92 patients with and without subgroup characterisation, but information was only available for 80 and 72 of them, respectively.

TABLE II. RSV Subgroups in Santa Fe (1993–1996)

Year	Total RSV	Analysed sample	Subgroup	
			A	B
1993	33	17	17 (100.0%)	0 (0%)
1994	40	8	4 (50.0%)	4 (50.0%)
1995	37	20	14 (70.0%)	6 (30.0%)
1996	67	40	38 (95.0%)	2 (5.0%)
Total	177	85	73 (85.9%)	12 (14.1%)

The proportion of children admitted to the hospital with RSV who were healthy previously compared with those with underlying conditions (known to predispose to more severe RSV infection) was analysed in relation to the subgroup of the infecting strain as another possible indicator of strain pathogenicity. Children with underlying conditions included those with cardiac and neurologic diseases, prematurity (<38 weeks gestation), malnourishment or history of perinatal respiratory pathologies. Of the infants admitted to the hospital with A strain infections, 35% had underlying conditions, as did 34% of those with B strain infections. Similarly, when each underlying condition was analysed separately, the difference in proportions was not statistically significant.

A significantly greater proportion of patients with Subgroup A infections had a feeding history (95% vs. 75% for A and B infections, respectively; Fisher's exact test, $P = 0.04$). This difference can not be explained by a difference in the mean ages of the two groups, because even after including the age of the patients in a logistic regression model, greater breast-feeding levels were statistically associated with Subgroup A infected children (adjusted odds ratio, 7.11, $P = 0.03$). Similarly, when only currently breast-fed children were considered as feeding, fewer children with Subgroup B infections were currently breast-feeding compared with children with Subgroup A infections (50% vs. 63% for B and A infections, respectively). This difference, however, was not statistically significant (Fisher's exact test, $P = 0.29$).

DISCUSSION

This 4-year retrospective study of children with ALRI and RSV diagnosis in Santa Fe City shows that there has been a predominance of Subgroup A infections. These findings agree with the data of Carballal et al. [1997] who reported Subgroup A as the strain found most frequently in Argentina from 1990 to 1996. Salomon et al. [1991] and Russi et al. [1989a] in Argentina and Uruguay respectively, however, showed that Subgroup B predominated in 1987 and 1988. In most years both subgroups occurred during the epidemic season as has been observed in previous studies originated in the Northern hemisphere [Akerlind and Norrby 1986; Hendry et al., 1986; Mufson et al., 1988].

This study found that A infections produced more severe disease than did B infections, as assessed on the length of hospital stay and use of respiratory support. The severity of illness was assessed by these two indirect measurements, given that monitoring by means of more objective parameters such as oxygen saturation and blood gases was feasible only in patients who were very ill. The length of stay can be used as a measure of severity, although it was not possible to control for interphysician differences in the decision to discharge the patient from hospital. The need for respiratory support seems a reliable indicator of severity. McIntosh et al. [1993], in a study of RSV outbreaks during 1989–91, however, found no correlation between strain group and the requirement of oxygen or ventilation. On the other hand, McConnochie et al. [1990] and Taylor et al. [1989] in analysing illnesses in infants hospitalised during two RSV outbreaks, one of predominantly Subgroup B and one of predominantly Subgroup A strains, suggested that the requirement of oxygen therapy or ventilation was suitable for discriminating between the

TABLE III. Clinical and Demographic Characteristics of Children Infected with RSV, According to Subgroup (Santa Fe, 1993–1996)

	Subgroup A (n = 73)	Subgroup B (n = 12)	*P* value[a]
Gender			
Males:Females	48:25	5:7	0.12 (F)
Mean age (months) (SD)	4.6 (3.6)	5.1 (3.4)	0.63 (T)
Illness			
Bronchiolitis	41	4	0.14 (P)[b]
Pneumonia	22	7	
Others (bronchitis, asthma)	10	1	
Days of evolution before admission (median)	3	4	0.25 (MW)
Score of severity[c,d]	75	7	0.01 (MW)
Age group <6 months	59 (49)[e]	6 (8)	0.03 (MW)
Age group >6 months	16 (19)	1 (4)	0.19 (MW)
Underlying conditions			
Yes	26	4	1.00 (F)
Breast feeding history[c]			
Positive	65	9	0.04 (F)

[a]F = Fisher's test; MW = Mann–Whitney test; T = Student's *t*-test; P = Pearson chi-squared.
[b]Comparison of the proportion of bronchiolitis among the groups.
[c]There were 73 patients with Subgroup A, but information was only available for 68 of them.
[d]Clinical score for the following indices, each assigned one point, (1) duration of hospital >5 days (2) respiratory support given.
[e]Numbers in parentheses, number of RSV subgroups identified in each age group.

severity of disease caused by each subgroup. Evidence of greater clinical severity associated with Subgroup A strains was obtained. In addition, Salomon et al. [1991] in Argentina, found that respiratory rate >50/min. and presence of atelectasis and wheezing were significantly more common among the population infected with Subgroup A strains. No correlation has been found, however, between oxygen saturation and the respiratory rate [Mulholland et al., 1990], that suggests that the latter may be of limited value as an indicator of the severity of the illness.

A greater proportion of children with Subgroup A infections had diagnosis of bronchiolitis in agreement with Mufson et al. [1988]. This association was not statistically significant in our study, however, possibly due to the small size of the sample studied.

The mean age of children infected with Subgroup A strains compare with those with Subgroup B strains, was examined on the premise that a significant difference may indicate differences in the duration of passive immunity or in pathogenicity between the two groups. The current study found that patients with Subgroup A infections tended to be slightly younger, although not significantly so, as has been observed in previous studies [Mufson et al., 1988; Russi et al., 1989b; Monto and Ohmit 1990]. On the other hand, the significantly smaller proportion of children with Subgroup B infections that had feeding history may suggest a lower virulence for Subgroup B strains.

Subgroup B infections seem to be less frequent in children admitted to the hospital. As Subgroup B strains may cause less severe illness, there may be the possibility that strains of this subgroup were recognised less frequently in children admitted to the hospital. Against this possibility, however, Mufson et al. [1988] found no difference between Subgroup A and Subgroup B in the proportion of children admitted from the community to the hospital for RSV infection. Hall et al. [1990] obtained, through routine home visits, nasal washings from young children with minimal respiratory signs. The proportion of A and B subgroups in these children compared with those from inpatients and outpatients children was found to be almost the same for each of 5 years.

During the recent years, RSV strains have been characterised by molecular techniques [Storch et al., 1993; Cane et al., 1994; Coggins et al., 1998; Hornsleth et al., 1998]. Thus, recent reports use PCR to type and go beyond to genotype to variants of Subgroups A and B [Coggins et al., 1998; Hornsleth et al., 1998]. Thus, Hornsleth et al. [1998] found that the severity of disease in patients admitted with acute RSV infections could be correlated to RSV genotype and type; they reported the uncommon evidence of greater clinical severity associated with group B strains, as assessed on the length of the hospital stay, use of respiratory support and the presence of an infiltrate on a chest radiograph.

Our findings, though limited in scope, suggest that a greater clinical severity of disease in patients admitted with acute RSV infections can be correlated to Subgroup A strains as determined by a serogrouping method. Additional studies, over a longer period, analysing the genetic variability among Group A and Group B RSV, may identify pathogenic strains that may help to explain the great variability in severity of the infections caused by RSV.

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